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Analytical and Clinical Utility of a Photometric Assay for Blood Coagulation Factor XIII

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Summary: A new photometric assay for factor XIII was evaluated for its analytical performance and clinical usefulness. The test showed good performance characteristics: intra-assay coefficients of variation between 0.83 and 2.68%, inter-assay coefficients of variation from 3.4 to 4.5%. The test can be conducted rapidly on an automated analyser such as the Cobas Bio®. The reference values (mean ± 2 SD) ranged from 67 to 147% and there was no gender difference. The comparability of the photometric test with a clot lysis factor XIII test showed an acceptable coefficient of correlation $r = 0.87$ ($p < 0.0001$). The diagnostic conformity of both tests was 76.7%. Factor XIII concentrations were assessed in seven patient groups. In liver cirrhosis, M. *Crohn* and during pregnancy noticeable percentages of lowered values were found: i.e. 18.2%, 11.8% and 10.0% respectively. Elevated values were seen in hypertensive patients (16%) and in the small group of patients with carcinoma of the ovarium (22.2%). These results show that the incidence of acquired factor XIII deficiencies is relative low. The clinical meaning of reduced or enhanced factor XIII needs to be clarified by more extensive patient studies.

Introduction

Coagulation factor XIII is a zymogen (proenzyme), present in the circulation, which is transformed into its active form (factor XIIIa) by the proteolytic attack of thrombin in the presence of Ca^{2+}. It is a transglutaminase (EC 2.3.2.13) catalysing an acyl transfer reaction. When a peptide-bound lysine residue is the acyl receptor, it cross-links two peptide chains (1).

The best known function of factor XIII is the cross-linking of fibrin to produce insoluble fibrin polymers (2). It simultaneously incorporates α_2-antiplasmin into fibrin, thereby reducing the initial fibrinolytic activity (3). Moreover, factor XIII promotes the cross-linking of a fibronectin with fibrin (3). The interaction of factor XIII with collagen results in an acceleration of wound healing (4) and stimulates fibroblast proliferation (5, 6). In addition to its presence in plasma, factor XIII also occurs in platelets and placenta. Thrombocytic and placental factor XIII might be identical (7). Plasmatic factor XIII consists of two "A" subunits complemented with a S unit, whereas thrombocytic and placental factor XIII consist of two subunits "A" (8). Many attempts have been made to determine factor XIII and to introduce such determinations into laboratory diagnosis. For this purpose, several assays for factor XIII have been developed (9–20). Most of them, however, are troublesome,

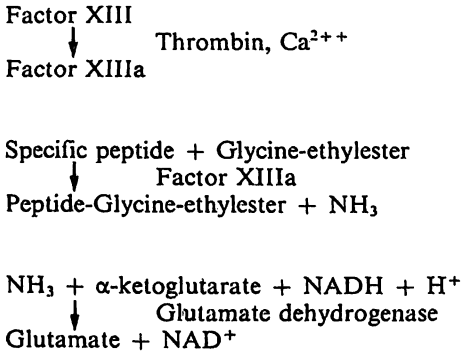

Fig. 1. Principle of the photometric factor XIII assay (The specific peptide is composed of ten amino acids with the sequence Leu-Gly-Pro-Gly-Gln-Ser-Lys-Val-Ile-Gly-amide).

poorly reproducible, time consuming and not suitable for automation. Recently the Behring Corporation developed a new assay for factor XIII activity measurement. The principle of the assay (see fig. 1) is the release of ammonia when factor XIIIa catalyses the coupling of glycine-ethylester with a new specific dekapeptide with a C-terminal amide group. The ammonia formed is determined from the decrease in absorbance at 340 nm due to dehydrogenation of NADH in the presence of glutamate dehydrogenase (EC 1.4.1.4) and 2-oxoglutarate. A peptide clot inhibitor is added to avoid interference due to gelation of the test mixture (21). In the present study we evaluated the analytical performance of the test, and applied it to a group of healthy volunteers and several patient groups.

Materials and Methods

Samples

Venous blood samples were collected from subjectively healthy individuals and from patients between 8.30 and 9.00 h. Citrated plasma was prepared by centrifuging 9 volumes of freshly drawn blood with 1 volume of trisodium citrate (0.11 mol/l) for 20 min at 1800 *g*. The plasma was used immediately or stored at −70 °C in plastic tubes and thawed with tap water at 37 °C for 5 min before use.

Methods

For the quantitative factor XIII assay we used the test kit of Behring (Marburg, Germany). For comparison purposes we performed the clot lysis assay according to *Karges* (1), also purchased from Behring (Marburg, Germany). The results of the clot lysis test are given as the highest dilution (range 25% to 200% in steps of 25%) of plasma with a lysis resistant clot residue.

Test procedure

The determinations were performed on a Cobas Bio® centrifugal analyser (Hoffmann La Roche, Basel, Switzerland). The measuring conditions for the Cobas Bio are given in table 1.

Patients

The reference range was determined on 102 subjectively healthy volunteers (age range 20−50 years, mean age 38 years. The test was also performed on patients with liver cirrhosis (n = 25), patients with M. *Crohn* in the phase of exacerbation (n = 22), and on patients with hypertension (n = 51), with carcinoma of the lung (n = 48), of the colon (n = 23), of the ovarium (n = 9), and during pregnancy (n = 56). The same patients have been described in detail elsewhere (22−26).

The patients with liver cirrhosis consisted of nine patients with *Child-Turcotte* classification A, nine patients with *Child-Turcotte* classification B and 7 patients with *Child-Turcotte* classification C. The patients with M. *Crohn* had a "*van Hees*" activity index ranging from 210−313.

The hypertension patients consistently showed raised blood pressures: the diastolic pressure ranged between 90 and 144 mmHg; the systolic pressure varied from 130−200 mmHg.

Tab. 1. Cobas Bio measuring conditions for the photometric factor XIII assay.

1	Units	Own
2	Calculation factor	0
3	Standard 1 conc	99
4	Standard 2 conc	99
5	Standard 3 conc	99
6	Limit	0
7	Temperature (°C)	37.0
8	Type of analysis	2
9	Wavelength (nm)	340
10	Sample volume (µl)	25
11	Diluent volume (µl)	10
12	Reagent volume (µl)	250
13	Incubation time (s)	180
14	Start reagent volume (µl)	0
15	Time of first reading (s)	300.0
16	Time interval (s)	30
17	Number of readings	10
18	Blanking mode	1
19	Printout mode	2

The carcinoma patients were untreated and recently diagnosed. The gestation periods of the pregnant women varied from 6−40 weeks (mean 25.1 weeks).

Results

Reproducibility was tested at different factor XIII concentrations with samples obtained by mixing pooled normal plasma and factor XIII-deficient plasma or saline. The intra-assay coefficient of variation ranged from 0.83 to 2.68% depending on the tested concentration. The inter-assay coefficient of variation was 3.4% (at 95.8% factor XIII) and 4.5% at 55.1% factor XIII (see tab. 2).

Tab. 2. Reproducibility of the factor XIII assay (n.d. = not done).

F XIII concentration (%)	Intra-assay CV (%)	n	F XIII concentration (%)	Inter-assay CV (%)	n
120.0	0.83	20	n.d.	n.d.	–
91.7	1.35	20	95.8	3.4	10
53.3	2.68	20	55.1	4.5	10
39.1	1.08	20	n.d.	n.d.	–

Table 3 contains a contingency table of the photometric factor XIII results in comparison with those of the clot lysis factor XIII assay. Diagnostic confirmity was 76.7%. A *Spearman* rank coefficient of correlation was calculated of $r = 0.87$; $p < 0.0001$.

The reference values (tab. 4) were calculated from 102 subjectively healthy individuals (46 males, 56 females). No differences were seen between the sexes. The average value was 107%, the median value 104%. Since

Tab. 3. Diagnostic conformity (76.7%) of the photometric and the clot lysis factor XIII tests (n = 155).

		Number of samples at each factor XIII concentration determined by clot lysis		
		0–75%	75–125%	125–200%
Number of samples	0– 75%	8	20	0
at each factor XIII concentration	75–150%	0	107	16
determined by the photometric assay	>150%	0	0	4

Tab. 4. Basic statistics and reference range of a group of healthy volunteers.

	Factor XIII plasma concentration
Average	107%
Median	104%
Standard deviation (SD)	20%
Lower quartile	91%
Upper quartile	123%
Interquartile range	31%
Reference range (mean ± 2 SD)	67–147%

the distribution was *Gaussian*, the reference values were taken as mean value ± 2 SD i.e. 67–147%.

In table 5 the results of the factor XIII concentrations in different disease states are given. High percentages of low values were found in liver cirrhosis (18.2%), M. *Crohn* (11.8%), carcinoma of the ovarium (22.2%) and during pregnancy (10.0%). High proportions of elevated values were seen in hypertensive patients (16%) and in carcinoma of the ovarium (22.2%).

Discussion

The newly available kinetic factor XIII assay showed good reproducibility, comparable to that reported in the literature (27). Comparability with a clot stability test was also satisfactory and in close agreement with other results (27). The reference values were also in concordance with those reported in the literature (27). The new test shows good performance characteristics; it is also rapid, easy to perform and shows good reproducibility. With regard to the clinical usefulness of the test, we know from the literature that a congenital factor XIII deficiency is the exception, whereas the acquired form is relatively common. As early as 1972, a substantial decrease was reported in patients with acute leukaemia (28). Another case of factor XIII deficiency was reported in 1982 by *Kuratsuji* et al. (29) in antibiotic-associated pseudo-membranous colitis. A successful pregnancy of a woman with congenital factor XIII deficiency treated with substitutive therapy has been described by *Rodeghiero* et al. (30) and *Boda* et al. (31). Several studies have dealt with low factor XIII concentrations in *Crohn*'s disease (32) and colitis ulcerosa (33) and the treatment of patients suffering from these diseases with factor XIII concentrate (34, 35). Most recently, factor XIII deficiencies were reported in systemic haematological disorders, morbus *Schönlein Hennoch* and bacterial infections, as well as in inflammatory bowel disease (36). Regarding the clinical usefulness of the test, we conclude from the results of the present study that the photometric factor XIII test is useful for the detection of moderate deficiencies as established in patients with

Tab. 5. Values for factor XIII in several patient groups.

Patient groups		F XIII concentrations (%)					
		$\bar{x}$	SD	Min	Max	FXIII decreased[1])	FXIII increased[2])
Liver cirrhosis	(n = 25)	97.0	29.6	36	166	18.2	3.0
M. *Crohn* in acute phase with thrombocytosis	(n = 22)	100.0	22.1	47	136	11.8	0
Hypertension	(n = 51)	110.6	24.6	72	173	9	16.0
Carcinoma of the lung	(n = 48)	104.4	26.4	58	163	4.7	7.0
Carcinoma of the colon	(n = 23)	102.7	17.8	65	146	4.3	4.3
Carcinoma of the ovarium	(n = 9)	110.1	39.9	56	183	22.2	22.2
Pregnancy	(n = 56)	93.7	19.6	54	136	10.0	0

[1]) percentage of values beneath the lower limit of the reference range.
[2]) percentage of values above the upper limit of the reference range.

liver cirrhosis, morbus *Crohn* and during pregnancy. It must be considered that the evaluation of protein changes during pregnancy is complicated by the redistribution of body water with increasing gestational age, which might cause a lowering of protein concentrations. In the present study, however, factor XIII did not show a significant decrease with ongoing pregnancy ($r = -0.026$, n.s.). Nevertheless, lowered factor XIII during pregnancy may be partly due to the described effect.

The detection of elevated factor XIII might also be of clinical value, e.g. in hypertensive patients and perhaps in patients with carcinoma of the ovarium, although this group is too small to draw definitive conclusions. The demonstrated incidence of decreased or increased values, however, seems to be relatively low. The clinical significance of the observed raised or lowered factor XIII values is therefore not clear. More extensive clinical studies are required to address these issues.

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